ClinGen *CDH1* Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 3.1
This version specified for the following genes: *CDH1*
Expert Panel Page: https://www.clinicalgenome.org/affiliation/50014

Gene	Disease (MONDO ID)	Clinically significant transcript
CDH1	**hereditary diffuse gastric cancer (MONDO:0007648)**	**NM_004360.5**

Summary of changes in Version 3.1 (March 2022):

(1) Specification of PM5_Supporting to nonsense and frameshift variants that are predicted/proved to undergo nonsense-mediated decay (NMD) or located upstream of the last known pathogenic truncating variant [c.2506G>T (p.Glu836Ter)].

(2) Column correction for PM2_Supporting from Moderate column to Supporting column.

ACMG/AMP Criteria Codes	Original ACMG/AMP Rule Summary	*CDH1* Rule Specifications					
		Stand Alone	**Very Strong**	**Strong**	**Moderate**	**Supporting**	**Comments**
PVS1	Null variant in a gene where LoF is a known mechanism of disease	---	Per modified *CDH1* PVS1 decision tree	Per modified *CDH1* PVS1 decision tree Other *CDH1* caveats: - Use PVS1_Strong as the default strength of evidence for canonical splice site variants and follow the site-specific recommendations in the splicing table. - *CDH1* Exonic deletions or tandem duplications of in-frame exons (exon 4,5,8,9,12,13,15)	Per modified *CDH1* PVS1 decision tree Other *CDH1* caveats: - G to non-G variants disrupting the last nucleotide of an exon - Canonical splice sites predicted or demonstrated experimentally to result in in-frame partial skipping/insertion (e.g., Exon 3 donor site)	N/A	RNA analysis is recommended for splicing alterations, and if the RNA evidence does not support the prediction, the strength should be updated

PS1	Same amino acid change as a previously established pathogenic variant regardless of nucleotide change	---	---	---	---	---	Not applicable for *CDH1*
PS2	*De novo* (both maternity and paternity confirmed) in a patient with the disease and no family history	---	≥Two patients meet the HDGC individual phenotype criteria w/ parental confirmation	One patient meets the HDGC individual phenotype criteria w/ parental confirmation	---	---	Use ClinGen's *de novo* point system for a highly specific phenotype (see Table S2)
PS3	Well-established *in vitro* or *in vivo* functional studies supportive of a damaging effect on the gene or gene product	---	---	RNA assay demonstrating abnormal out-of-frame transcripts	---	RNA assay demonstrating abnormal in-frame transcripts	This rule can only be applied to demonstrate splicing defects.
PS4	Prevalence of variant in affected individuals is significantly increased compared to controls	---	≥Sixteen families meet HDGC criteria	Four - Fifteen families meet HDGC criteria	Two or three families meet HDGC criteria	One family meets HDGC criteria	Use the 2020 updated clinical practice guidelines (PMID: 32758476) as the HDGC phenotype criteria. PS4 cannot be applied to variants that meet BS1 or BA1, or to variants in which less than 30% of reported individuals meet HDGC criteria.
PM1	Located in a mutational hot spot and/or critical and well-established functional domain without benign variation	---	---	---	---	---	Not applicable for *CDH1*
PM2	Absent in population databases	---	---	---	---	≤ One out of 100,000 alleles in gnomAD cohort; if	Use gnomAD to determine allele frequency. The mean

						present in ≥2 individuals within a subpopulation, must be present in ≤ One out of 50,000 alleles	coverage of *CDH1* in the population database used should be at least 30x.
PM3	For recessive disorders, detected in trans with a pathogenic variant	---	---	---	---	---	Not applicable for *CDH1*
PM4	Protein length changes as a result of in-frame deletions/insertions in a nonrepeat region or stop-loss variants	---	---	---	Only apply to stop-loss variants Variant example: *CDH1* c.2647T>C (p.Ter883Glnext*29)	---	PM4 is not applied to small in-frame indels because the impact of amino acid level changes of *CDH1* variants is inconclusive.
PM5	Novel missense change at amino acid residue where a different missense variant is pathogenic	---	---	---	---	PM5_supporting is applicable to nonsense and frameshift variants that are predicted/proved to undergo NMD or located upstream of the last known pathogenic truncating variant. Site-specific recommendations for the application of PM5_Supporting for canonical splicing variants	The nonsense or frameshift variant must not impact splicing based on RNA assay or splicing predictions.

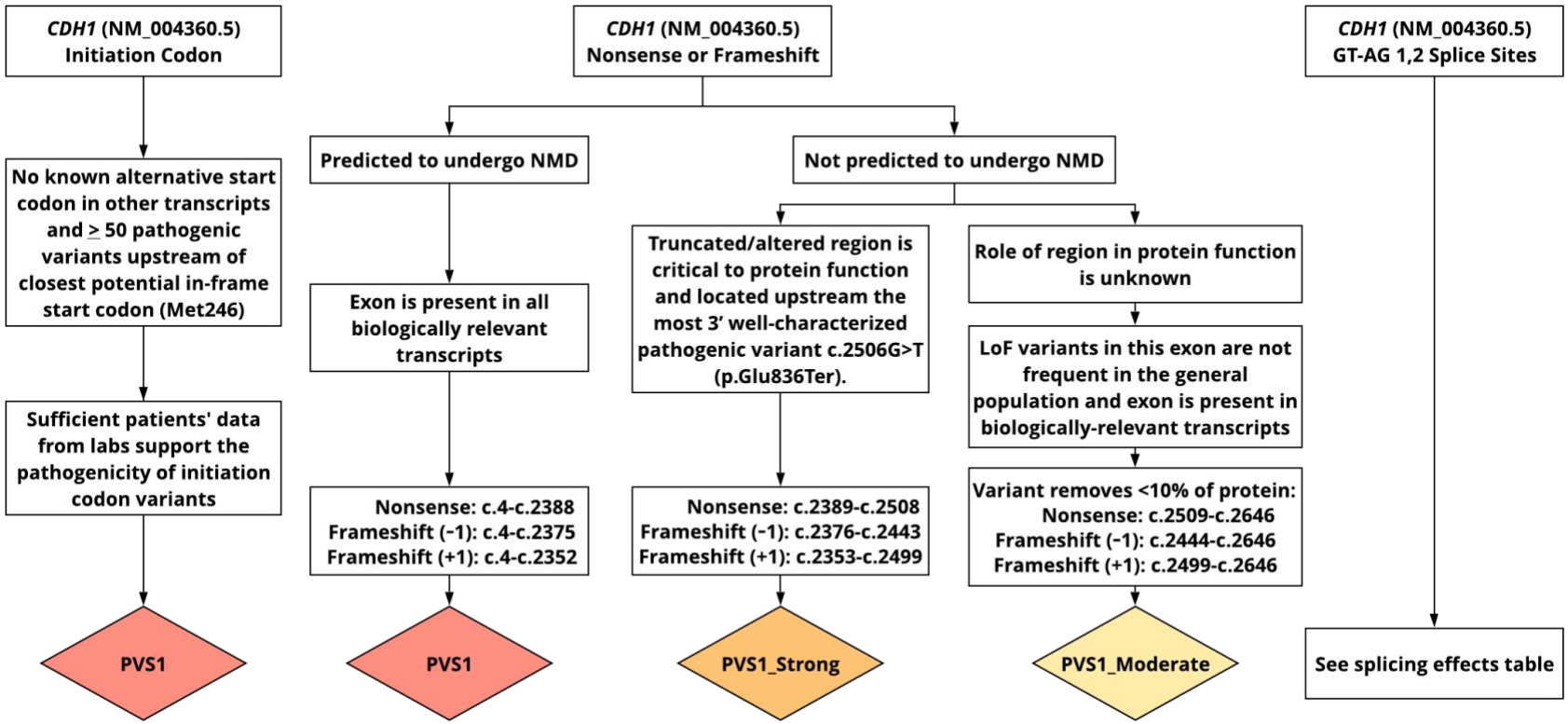
						are provided in the splicing table.	
PM6	Assumed *de novo*, but w/o confirmation of paternity and maternity	---	≥Four patients meet the HDGC individual phenotype criteria w/o parental confirmation	≥Two patients meet the HDGC individual phenotype criteria w/o parental confirmation	One patient meets the HDGC individual phenotype criteria w/o parental confirmation	---	Use ClinGen's *de novo* point system for a highly specific phenotype (See Table S2)
PP1	Cosegregation in multiple affected family members in a gene definitively known to cause the disease	---	---	≥Seven informative meioses across ≥2 families	Five-six informative meioses across ≥1 family	Three-four informative meioses across ≥1 family	Based strength of rule code on number of meioses across one or more families
PP2	Missense variant in a gene with a low rate of benign missense variation & where missense variants are a common mechanism of disease	---	---	---	---	---	Not applicable for *CDH1*
PP3	Multiple lines of computational evidence support a deleterious effect on the gene or gene product	---	---	---	Variants affecting the same splice site as a well-characterized variant with similar or worse *in silico*/ RNA predictions	At least three *in silico* splicing predictors in agreement (SpliceAI, MaxEntScan, SSF, GeneSplicer, HSF, TraP, varSEAK)	PP3 cannot be applied for canonical splice sites. PP3 code also does not apply to the last nucleotide of exon 3 (c.387G). Do not use protein-based computational prediction models for missense variants.
PP4	Patient's phenotype or family history is highly specific for a disease	---	---	---	---	---	Not applicable for *CDH1*

	with a single genetic etiology						
PP5	Reputable source recently reports variant as pathogenic	---	---	---	---	---	Not applicable for *CDH1*
BA1	Allele frequency is greater than expected for disorder	MAF cutoff of 0.2%	---	---	---	---	99.99% CI; subpopulation must ≥ 2,000 alleles and have a minimum of five variant alleles present
BS1	Allele frequency is greater than expected for disorder	MAF cutoff of 0.1%	---	---	---	---	99.99% CI; subpopulation must ≥ 2,000 alleles and have a minimum of five variant alleles present. We allow a variant to reach a likely benign classification based on BS1 alone.
BS2	Observed in a healthy adult individual for a dominant disorder with full penetrance expected at an early age	---	---	Variant seen in ≥10 individuals w/o GC, DGC, gSRC tumors, or LBC & whose families do not suggest HDGC	---	Variant seen in ≥3 individuals w/o GC, DGC, SRC tumors, or LBC & whose families do not suggest HDGC	We allow a variant to reach a likely benign classification based on BS2 alone. BS2 cannot be applied to variants in which more than 30% of reported individuals meet HDGC criteria.
BS3	Well-established *in vitro* or *in vivo* functional studies show no damaging effect on protein function or splicing	---	---	Functional RNA studies demonstrating no impact on transcript composition	---	---	This rule can only be used to demonstrate lack of splicing and can only be applied to Synonymous, Intronic or Non-coding variants. BS3 may be downgraded based on quality of data

BS4	Lack of segregation in affected members of a family	---	---	Per original ACMG/AMP guidelines	---	---	Beware of the presence of phenocopies (e.g., breast cancer) that can mimic lack of segregation. Also, families may have more than one pathogenic variant contributing to another AD disorder
BP1	Missense variant in a gene for which primarily truncating variants are known to cause disease	---	---	---	---	---	Not applicable for *CDH1*
BP2	Observed in a healthy homozygous individual, or in *trans* with a pathogenic variant for a fully penetrant dominant gene/disorder or observed in *cis* with a pathogenic variant	---	---	Variant observed *in trans* w/known pathogenic variant (phase confirmed) **OR** observed in the homozygous state in individual w/o personal &/or family history of DGC, LBC, or SRC tumors	---	Variant is observed *in cis* (or phase is unknown) w/ a pathogenic variant **OR** observed in the homozygous state in gnomAD	Evidence code is dependent on the strength of data. Take consideration of the quality of sequencing data when applying code.
BP3	In-frame deletions/insertions in a repetitive region without a known function	---	---	---	---	---	Not applicable for *CDH1*

BP4	Multiple lines of computational evidence suggest no impact on gene/gene product	---	---	---	---	Splicing predictions only. At least three *in silico* splicing predictors in agreement (SpliceAI, MaxEntScan, SSF, GeneSplicer, HSF, TraP, varSEAK)	Do not use protein based computational prediction models and BP4 is not applicable for missense variants.
BP5	Variant found in a case with an alternate molecular basis for disease	---	---	---	---	Per original ACMG/AMP guidelines	This applies if a P/LP variant is identified in an alternate gene known to cause HDGC (currently only *CTNNA1*)
BP6	Reputable source recently reports variant as benign	---	---	---	---	---	Not applicable for *CDH1*
BP7	Synonymous variant which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site & the nucleotide is not highly conserved.	---	---	---	---	Synonymous and intronic variants at or beyond +7 to -21 locations.	Note the *CDH1* rule specification does not require a conservation prediction. We allow use of BP7 with BP4, as appropriate, to classify variants meeting both criteria as likely benign.

PVS1 decision tree



Splicing Table

Exon	GT-AG 1,2 Splice site	Location	Prediction if exon skipping	RNA assay	Canonical splice site variants curated	Recommended PVS1 strength and the application of PM5_Supporting criterion
Exon 1	Donor	c.48	Likely cryptic site		LP: c.48+1G>A	PVS1_Strong+PM5_Supporting
Exon 2	Acceptor	c.49	frameshift	frameshift	P: c.49-2A>G*	PVS1_Strong+PM5_Supporting
	Donor	c.163				PVS1_Strong
Exon 3	Acceptor	c.164	frameshift			PVS1_Strong
	Donor	c.387		in-frame transcripts	VUS: c.387+1G>A*	PVS1_Moderate
Exon 4	Acceptor	c.388	In-frame deletion (aa130-177)			PVS1_Strong
	Donor	c.531			LP: c.531+1G>A	PVS1_Strong+PM5_Supporting
Exon 5	Acceptor	c.532	In-frame deletion (aa178-229)		LP: c.532-1G>C	PVS1_Strong+PM5_Supporting
	Donor	c.687			LP: c.687+1G>A; c.687+2T>C	PVS1_Strong+PM5_Supporting
Exon 6	Acceptor	c.688	frameshift			PVS1_Strong
	Donor	c.832			P: c.832+1G>T LP: c.832+1G>A	PVS1_Strong+PM5_Supporting

Exon 7	Acceptor	c.833	frameshift	frameshift	P: c.833-2A>G*	PVS1_Strong+PM5_Supporting
	Donor	c.1008		frameshift with cryptic site	LP: c.1008+2T>C; c.1008G>A*; c.1008G>T*	PVS1_Strong+PM5_Supporting
Exon 8	Acceptor	c.1009	In-frame deletion (aa337-379)			PVS1_Strong
	Donor	c.1137		frameshift with cryptic site	P: c.1137+1delG; c.1137G>A* LP: c.1137+1G>A; c.1137+2T>C	PVS1_Strong+PM5_Supporting
Exon 9	Acceptor	c.1138	In-frame deletion (aa380-440)			PVS1_Strong
	Donor	c.1320		in-frame deletion (exon 9 skipping)	LP: c.1320+1G>C*	PVS1_Strong+PM5_Supporting
Exon 10	Acceptor	c.1321	frameshift			PVS1_Strong
	Donor	c.1565			P: c.1565+1G>C; c.1565+1G>A; c.1565+1G>T; c.1565+2dupT LP: c.1565+1delG	PVS1_Strong+PM5_Supporting
Exon 11	Acceptor	c.1566	frameshift	predicted in-frame insertion with potential rescue transcript	VUS: c.1566-1G>C; c.1566-2A>G	PVS1_Moderate
	Donor	c.1711			LP: c.1711+1G>C; c.1711+1G>A; c.1711+2_1711+7delTAAGGG	PVS1_Strong+PM5_Supporting

Exon 12	Acceptor	c.1712	In-frame deletion (aa571-646)	in-frame deletion (c.1712_1720del9)	VUS: c.1712-2A>C*	PVS1_Moderate
	Donor	c.1936				PVS1_Strong
Exon 13	Acceptor	c.1937	In-frame deletion (aa646-722)			PVS1_Strong
	Donor	c.2164			VUS: c.2164+2T>C VUS: c.2164+2dup (this variant actually affects +3 location)	PVS1_Strong
Exon 14	Acceptor	c.2165	frameshift		LP: c.2165-1G>C	PVS1_Strong+PM5_Supporting
	Donor	c.2295				PVS1_Strong
Exon 15	Acceptor	c.2296	In-frame deletion (aa766-813)		LP: c.2296-1G>A; c.2296-2A>G	PVS1_Strong+PM5_Supporting
	Donor	c.2439				PVS1_Strong
Exon 16	Acceptor	c.2440	Likely cryptic site	abnormal splicing	LP: c.2440-2A>G*	PVS1_Strong+PM5_Supporting

*** RNA functional assay performed.**

Application of PM5_Supporting to nonsense/frameshift variants that are predicted/proved to undergo NMD.

Note: PM5_Supporting can be applied to truncating variants located upstream of the last known pathogenic truncating variant [c.2506G>T (p.Glu836Ter)].

Exon	Location	No. of nonsense/frameshift curated (N=113) before applying PM5_Supporting
Exon 1	c.1-c.48	P (1): c.26C>A (p.Ser9Ter) LP (2): c.11G>A (p.Trp4Ter); c.12G>A (p.Trp4Ter)
Exon 2	c.49-c.163	P (4): c.59G>A (p.Trp20Ter); c.60G>A (p.Trp20Ter); c.70G>T (p.Glu24Ter); c.124_126delCCCinsT (p.Pro42Serfs) LP (1): c.76G>T (p.Glu26Ter)
Exon 3	c.164-c.387	P (8): c.187C>T (p.Arg63Ter); c.208dup (p.Ser70Phefs); c.220C>T (p.Arg74Ter); c.283C>T (p.Gln95Ter); c.308G>A (p.Trp103Ter); c.360dup (p.His121fs); c.377del (p.Pro126Argfs); c.382delC (p.His128Ilefs) LP (5): c.202delT (p.Tyr68Ilefs); c.261delG (p.Arg87Serfs); c.315delC (p.Thr106Profs); c.337A>T (p.Lys113Ter); c.369_375CCGCCCC[3] (p.His128fs)
Exon 4	c.388-c.531	P (6): c.454_460delCAGAAGA (p.Gln152Glufs); c.480_486delinsAGAATA (p.Ile161fs); c.489C>A (p.Cys163Ter); c.521dupA (p.Asn174Lysfs); c.504delA (p.Gly169Alafs); c.529C>T (p.Gln177Ter) LP (7): c.436_437TC[1] (p.Pro147fs); c.454_460dup (p.Arg154Thrfs); c.455_465delAGAAGAGAGAC (p.Gln152Leufs); c.457_460delAAGA (p.Lys153Glufs); c.457A>T (p.Lys153Ter); c.467G>A (p.Trp156Ter); c.468G>A (p.Trp156Ter)
Exon 5	c.532-c.687	P (2): c.603delT (p.Val202Leufs); c.656del (p.Pro219fs) LP (1): c.594_595insT (p.Thr199fs)
Exon 6	c.688-c.832	P (1): c.720del (p.Asn240fs) LP (4): c.692_693TC[2] (p.His233fs); c.707C>A (p.Ser236Ter); c.781G>T (p.Glu261Ter); c.793G>T (p.Glu265Ter)
Exon 7	c.833-c.1008	P (2): c.940A>T (p.Lys314Ter); c.1003C>T (p.Arg335Ter)
Exon 8	c.1009-c.1137	P (7): c.1009_1010delAG (p.Ser337Phefs); c.1023T>G (p.Tyr341Ter); c.1051C>T (p.Gln351Ter); c.1064delT (p.Leu355Terfs); c.1085delT (p.Val362Glyfs); c.1107del (p.Asn369Lysfs); c.1131del (p.Thr378fs) LP (1): c.1031_1032dup (p.Val345fs)

Exon 9	**c.1138-c.1320**	**P (2): c.1147C>T (p.Gln383Ter); c.1235_1236TA[3] (p.Ile415fs)** **LP (2): c.1170del (p.Asn390fs): c.1312del (p.Thr438fs)**
Exon 10	**c.1321-c.1565**	**P (4): c.1408del (p.Thr470fs); c.1476_1477delAG (p.Arg492Serfs); c.1488_1494delCGAGGAC (p.Glu497Leufs); c.1531C>T (p.Gln511Ter)** **LP (7): c.1341del (p.Lys447fs); c.1354_1357del (p.Leu452fs); c.1390del (p.Val464fs); c.1443del (p.Asn481fs); c.1460_1461del (p.Val487Alafs); c.1480G>T (p.Glu494Ter); c.1505delG (p.Gly502Alafs)**
Exon 11	**c.1566-c.1711**	**P (4): c.1578G>A (p.Trp526Ter); c.1587dup (p.Ala530fs); c.1590dup (p.Asn531fs); c.1612delG (p.Asp538Thrfs)** **LP (3): c.1569T>A (p.Tyr523Ter); c.1636delG (p.Ala546Leufs); c.1679dup (p.Tyr561Valfs)**
Exon 12	**c.1712-c.1936**	**P (6): c.1733dup (p.Gly579fs); c.1779dup (p.Ile594fs); c.1792C>T (p.Arg598Ter); c.1895_1896delAC (p.His632Argfs); c.1913G>A (p.Trp638Ter); c.1921C>T (p.Gln641Ter)** **LP (2): c.1746dup (p.Leu583Alafs); c.1917_1918del (p.Ile640fs)**
Exon 13	**c.1937-c.2164**	**P (5): c.1979dup (p.Gly661_Asp662insTer); c.1999del (p.Leu667fs); c.2062_2063TG[1] (p.Cys688_Glu689delinsTer); c.2095C>T (p.Gln699Ter); c.2100del (p.Val701Serfs);** **LP (8): c.1942G>T (p.Glu648Ter); c.1948_1949del (p.Ile650HisfsTer12); c.1948_1949dup (p.Ile651Serfs); c.1993del (p.Ile665Serfs); c.2029dup (p.Gln677Profs); c.2076_2077del (p.Gly693ArgfsTer3); c.2104G>T (p.Glu702Ter); c.2144delG (p.Gly715Glufs)**
Exon 14	**c.2165-c.2295**	**P (4): c.2265T>A (p.Tyr755Ter); c.2276delG (p.Gly759Glufs); c.2287G>T (p.Glu763Ter); c.2293C>T (p.Gln765Ter)** **LP (1): c.2272G>T (p.Glu758Ter)**
Exon 15	**c.2296-c.2439**	**P (2): c.2398delC (p.Arg800Alafs); c.2430delT (p.Phe810Leufs)** **LP (3): c.2311C>T (p.Gln771Ter); c.2324delG (p.Gly775Alafs); c.2386dup (p.Arg796Profs)**
Exon 16	**c.2440-c.2649**	**P (1): c.2506G>T (p.Glu836Ter)** **LP (2): c.2446A>T (p.Lys816Ter); c.2490dup (p.Leu831Alafs)** **VUS (5): c.2505_2506dup (p.Glu836fs); c.2526delT, p.(Ala843Leufs); c.2547_2548insA (p.Ser850fs); c.2549_2550delCC (p.Ser850Phefs); c.2594G>A (p.Trp865Ter)**

2020 hereditary diffuse gastric cancer (HDGC) genetic testing criteria

Family criteria, where family members are first-degree or second-degree blood relatives of each other:	
1	**≥2 cases of gastric cancer in family regardless of age, with at least one DGC**
2	**≥1 case of DGC at any age in family, and ≥1 case of LBC at age <70 years, in different family members**
3	**≥2 cases of LBC in family members <50 years of age**
Individual criteria:	
4	**DGC at age <50 years**
5	**DGC at any age in individuals of Maori ethnicity**
6	**DGC at any age in individuals with a personal or family history (first-degree relative) of cleft lip or cleft palate**
7	**History of DGC and LBC, both diagnosed <70 years**
8	**Bilateral LBC, diagnosed at age <70 years**
9	**Gastric in situ signet ring cells (SRC) or pagetoid spread of SRCs in individuals <50 years of age**